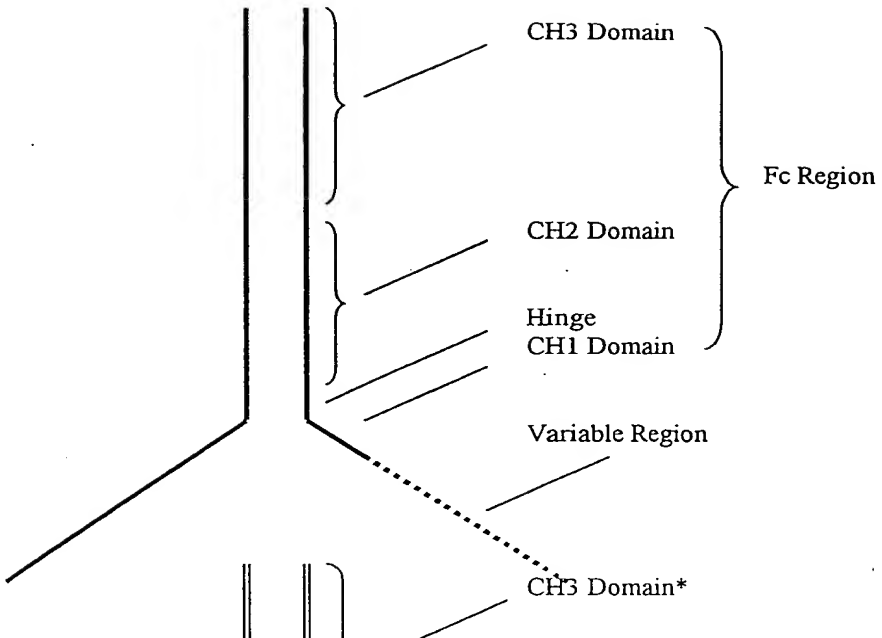

CH3 Domain
Fc Region
CH2 Domain
Hinge
CH1 Domain
Variable Region

Fig. 1A

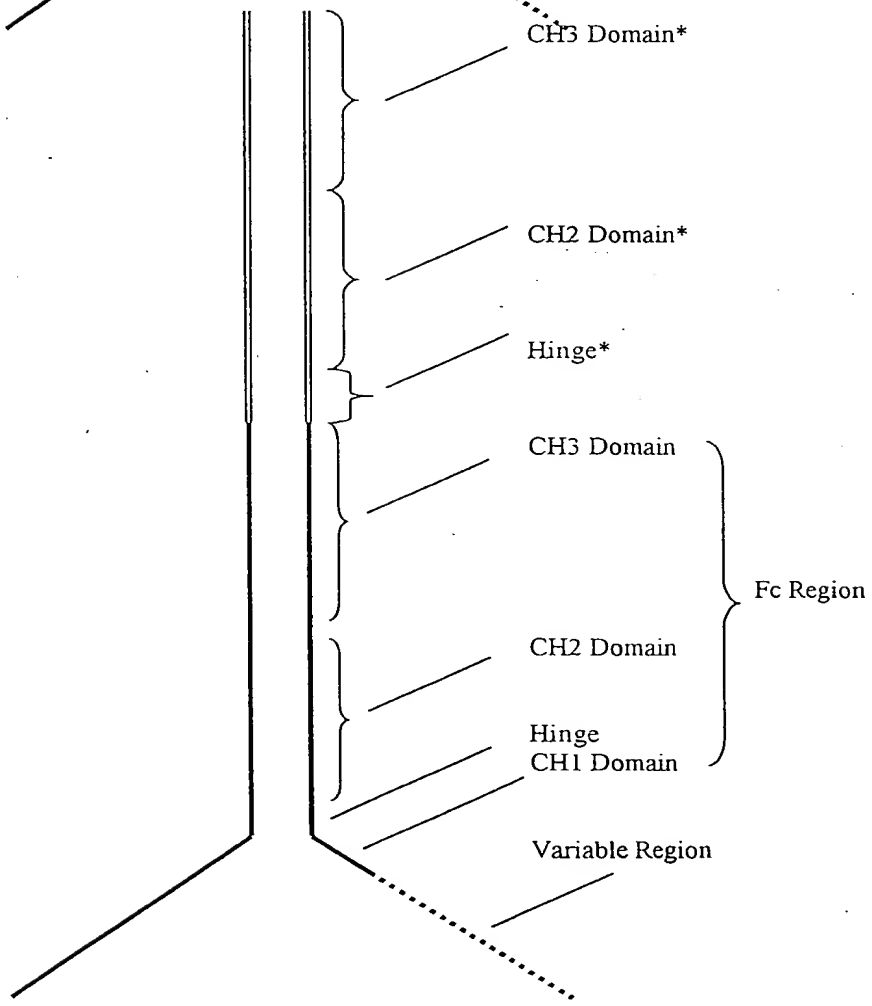

CH3 Domain*
CH2 Domain*
Hinge*
CH3 Domain
Fc Region
CH2 Domain
Hinge
CH1 Domain
Variable Region

Fig. 1B

# Fig. 2

Step 1: Creation of unique Bsu36I restriction (CCTTAGG) site in 3' terminus of gamma-4 constant region:

Sequence encoding last 4 amino acids of human gamma-4 constant region:

TCT CTG GGT AAA

Modified sequence encoding the same amino acids:

TCC TTA GGG AAG

Step 2: PCR primers utilized for accomplishing such modification:

Primer 1:

5'γ4 oligo GGG ACC CAC GGG GTG CGA GGG C (Dra III)

Primer 2:

3'γ4 oligo CTT CCC TAA GGA CAT GGA GAG GCT CTT CTG TGT GTG (Bsu36I)

Primer 3:

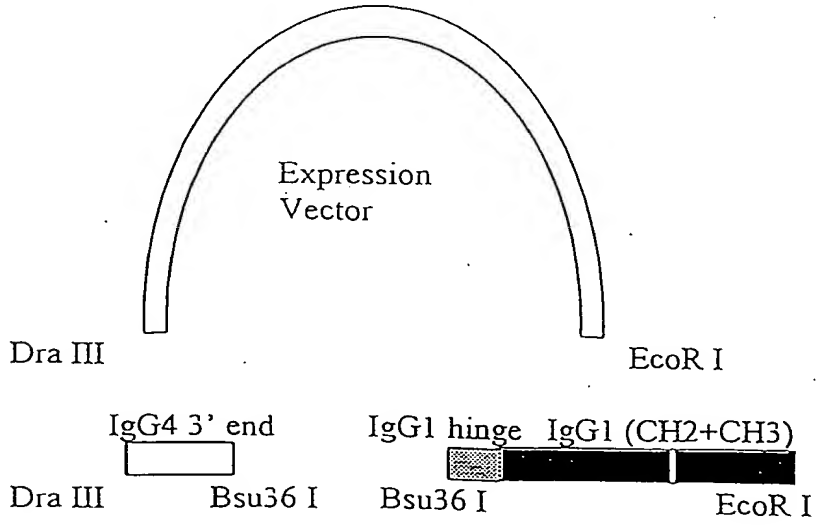
5'γ1oligo GAT TCC TTA GGG AAG GCA GAG CCC AAA TCT AGT GAC (Bsu36I)
ser

Primer 4:

3'γ1 oligo GCC GGA ATT CGG TAC GTG CCA AGC ATC CTC GTG C (EcoR I)

Step 3: Three way ligation:

a. introduce new Bsu36I site at gamma 4 -- hinge junction
b. add hinge and gamma 1 CH2 and CH3 domains
c. clone into DraIII-EcoRI sites of expression vector (VDJ-IgG4)



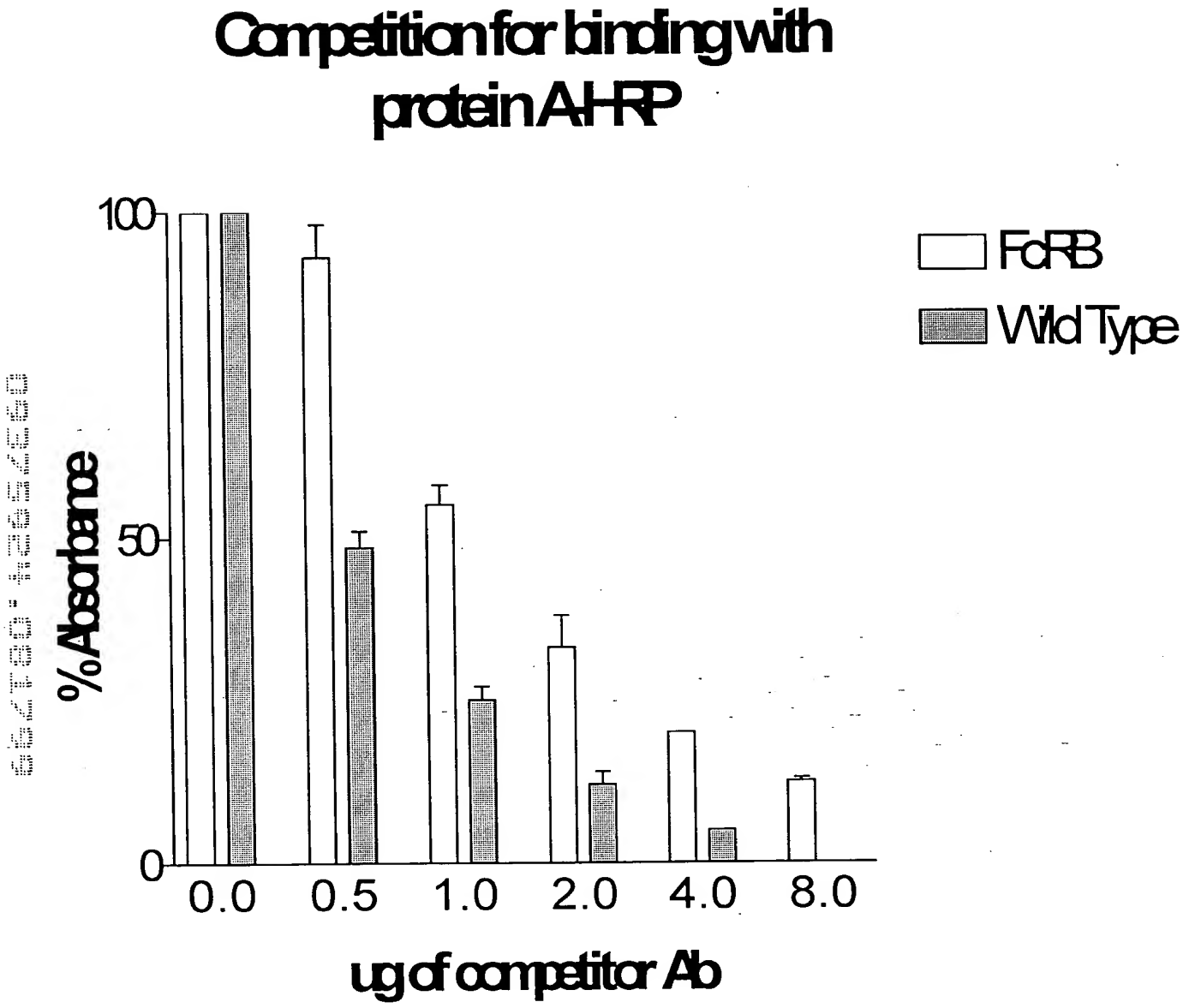

Competition for binding with protein A-HRP
100
50
0
% Absorbance
FcRB
Wild Type
0.0
0.5
1.0
2.0
4.0
8.0
ug of competitor Ab

FIG. 3